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Novel insights on acute myocarditis in pediatric patients

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Abstract. – Acute myocarditis (AM) is an inflammatory affliction of the heart muscle characterized by recent onset with a broad spectrum of clinical manifestations that globally affect millions of individuals, notably children and young adults.

The absence of distinct patterns of onset or predictable progression poses a significant threat to survival, potentially leading to advanced heart failure and malignant arrhythmias.

Myocardial fibrosis, a hallmark of myocardial remodeling, is increasingly recognized as a contributor to adverse outcomes in acute myocarditis cases. Advances in molecular and immunological techniques have highlighted the intricate interplay between viral infections, dysregulated immune responses, and genetic susceptibility.

Currently, there is no clear consensus for diagnosis or ongoing follow-up in pediatric patients. The conventional diagnostic tool, endomyocardial biopsy (EMB), considered the gold standard, has been complemented by the effectiveness of cardiac magnetic resonance imaging (CMRI) techniques. Given the procedural complexities and associated complications, there is a pressing need to explore non-invasive alternatives. In this context, biomarkers emerge as promising contenders by evaluating both the inflammatory processes and cardiac remodeling, providing valuable observations into disease severity, progression, and treatment response. Therapeutic strategies in these cases, focusing on the specific pathways or immune components associated with the etiologies, have exhibited promise for better outcomes.

Acute myocarditis in children remains a multifaceted clinical challenge, necessitating a comprehensive understanding of its pathophysiology, diagnosis, and management. This review aims to delve into novel insights surrounding the pathophysiology, diagnosis, and management of acute myocarditis in pediatric patients.

Key Words:

Biomarkers, Cardiac fibroblasts, Circulating miRs, Children, Fibrillar collagens, Heart dysfunction, inflammatory signals, Myocarditis, Myocardial fibrosis, TGF-β signaling.

Introduction

Acute myocarditis (AM) stands as an inflammatory myocardial injury characterized by a recent onset (<1 month), presenting a diverse range of clinical trajectories that often pose challenges in diagnosis. Its lack of specific onset patterns and unpredictable evolution can negatively impact prognosis through advanced heart failure and malignant ventricular tachyarrhythmias[1]. Globally, the prevalence of AM has been reported to range from 10.2 to 105.6 cases per 100,000 individuals, translating to an annual occurrence of approximately 1.8 million cases[2]. However, the accurate incidence and prevalence in the pediatric population are likely underestimated due to numerous cases not necessitating medical attention. Clinically, acute myocarditis constitutes about 0.05% of hospital admissions, predominantly affecting male adolescents with a prevalence of 81%[3]. The age distribution displays a bimodal pattern with peaks at 1 year and 16 years, while the most severe forms are observed in the initial year of life, extending up to the age of 4 and in adolescents[4]. Histological studies indicate myocarditis prevalence varying between 0.12% and 12%[5]. Notably, AM contributes to up to 46% of dilated cardiomyopathies and approximately 4% of heart failure cases, with a proportion of these cases associated with a poor prognosis[1,6,7]. In the age groups affected by myocarditis-related deaths, there has been a change with a shift from children and teenagers to adults as a result of the crucial role of healthcare systems in managing myocarditis[8]. In the pediatric population, is often challenging to es-

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tablish whether newly diagnosed heart failure or arrhythmias originate from an infectious trigger or are secondary to pre-existing cardiac conditions[9,10]. Currently, there is no consensus on the gold standard diagnostic approach for children. Histological verification, obtained through endomyocardial biopsy (EMB), is limited due to its high-risk nature and low specificity[11,12]. Cardiac magnetic resonance imaging (CMRI), an advanced imaging method, can be complicated by the requirement for deep sedation in young children and, in some cases, limited utility due to unstable arrhythmias[11].

This review aims to delve into novel insights surrounding acute myocarditis in pediatric patients.

Pathogenesis

While the etiology of acute myocarditis frequently remains undisclosed, confirmed cases, as indicated by the American Academy of Pediatrics, predominantly point towards an infectious or immune-mediated process as the underlying trigger, as shown in Figure 1.

Immune Cells and Inflammasome Molecular Mechanisms

Inflammation serves as a crucial defense mechanism in higher organisms, constituting the immune system's initial response. This response functions to eliminate injurious stimuli, such as infectious pathogens, damaged cells, or irritants, thus paving the way for the commencement of the healing process[13,14]. This fundamental process is intrinsic to all forms of myocardial injury[15]. The pathogenesis of myocarditis has been extensively elucidated through murine models, with these findings equally applicable to humans[16]. Among children, viral infection has emerged as the predominant cause of myocarditis[17], with compelling evidence underscoring the direct role of viral myocardial triggers and concurrent pathological host immune responses[15]. This intricate interplay occurs through a stepwise progression, as shown in Figure 2.

Furthermore, the type of virus involved determines the specific cardiac cells that are affected (Table I). For instance, Parvovirus B19 (Parvo B19) targets vascular endothelial cells and cardiomyocytes through interactions with the erythrocyte P antigen (P-Ag) and integrin

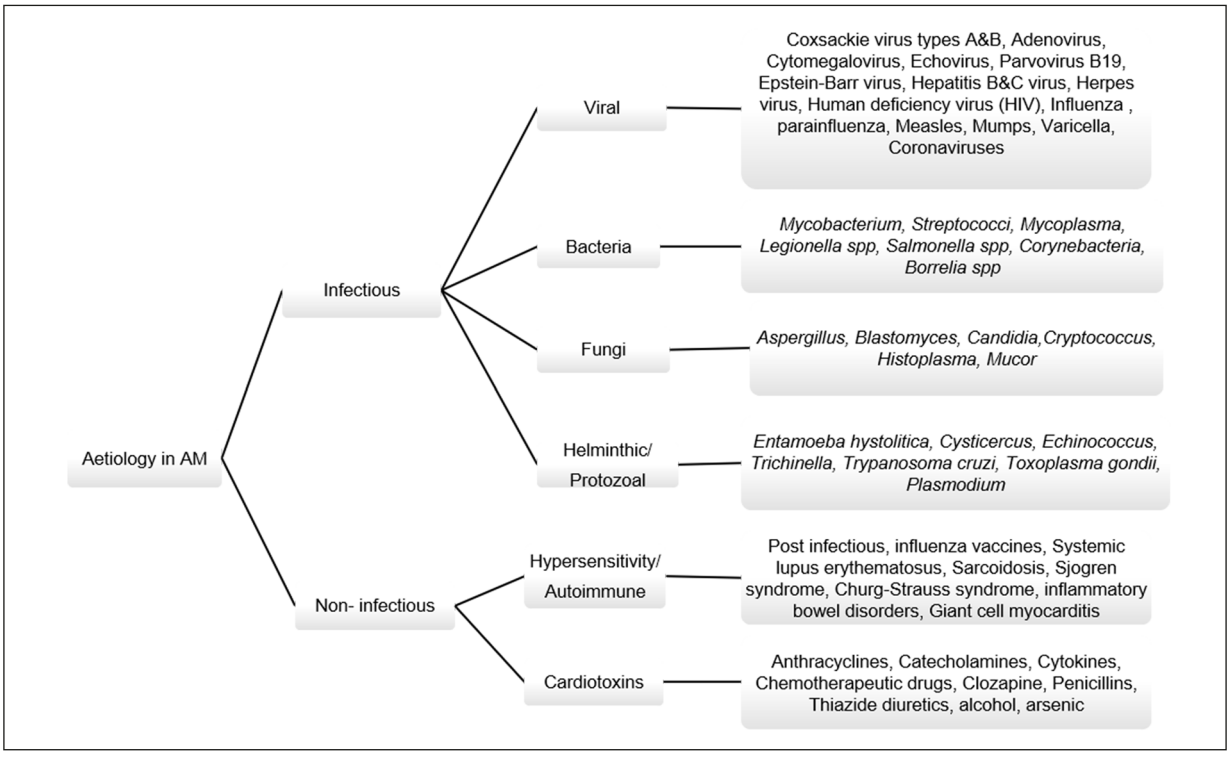

Figure 1. Diagram of major causes in AM.

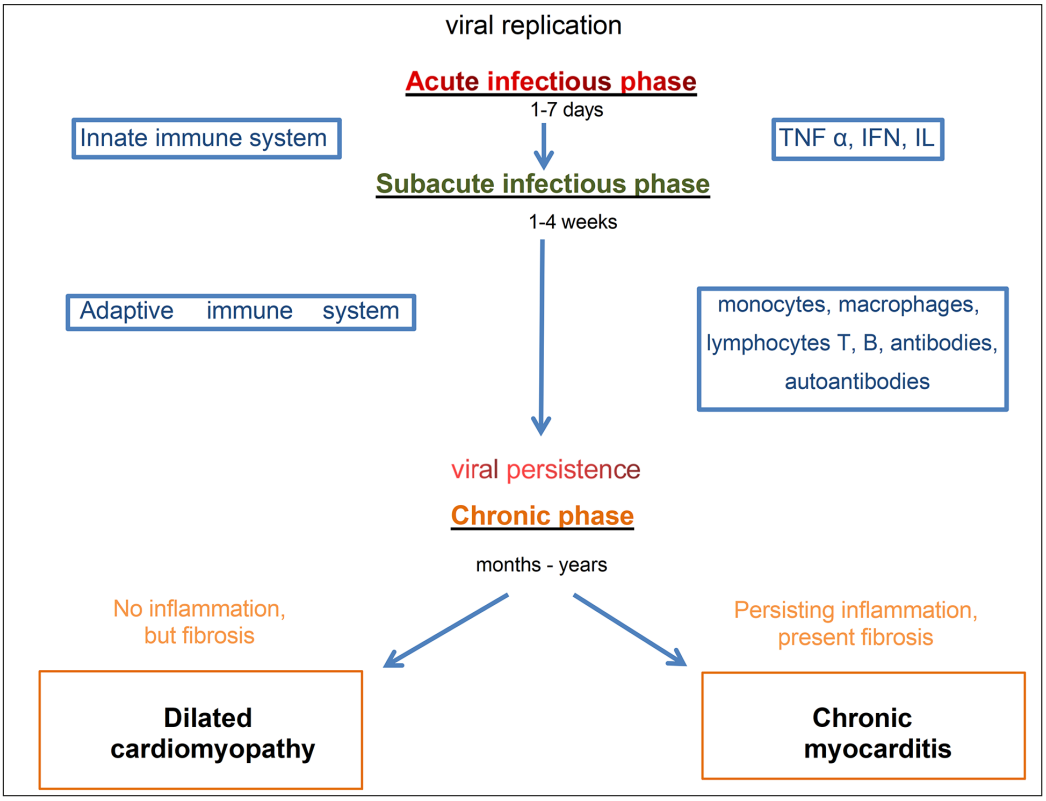

Figure 2. Three-phase model for the pathogenesis of viral myocarditis. TNF, tumour necrosis factor; IFN, interferon; IL, interleukin.

αvβ1 as a co-receptor. Human Herpetic Virus-6 (HHV-6) employs a cluster of differentiation (CD) 46 as a cellular receptor for CD4+ lymphocyte (Ly) T and endothelial cells. Enteroviruses, on the other hand, exert a direct cytolytic effect on cardiomyocytes. Coxsackie B and adenoviruses trigger cytokine synthesis and cell-mediated immune responses *via* the coxsackievirus and adenovirus receptor (CAR), utilizing the decay-accelerating factor (DAF) and integrins (αvβ3 and αvβ5) as additional receptors[17-22]. A suggested mechanism of viral invasion of SARS-CoV-2 is through angiotensin-converting enzyme 2 (ACE2) receptor, helped by a host transmembrane serine protease 2, targeting the myocytes[23].

Table I. Cardiotropic viruses and their target-cells in the pathogenesis of acute myocarditis.

Virus	Receptor/Co-receptor	Target cells	References
Parvo-B19	pAg, αvβ1	Endothelial cells cardiomyocytes	17,19-22
HHV-6	CD46, CD4+ LyT	Endothelial cells	
Coxsackie B Adenoviruses	CAR, DAF,αvβ3, αvβ5	Cardiomyocytes	
SARS-CoV-2	ACE2 receptor, transmembrane serine protease 2	Cardiomyocytes interstitial cells	23

(Parvo B-19) Parvovirus B19; (pAg) erythrocyte P antigen; (HHV-6) Human Herpetic Virus-6; (CD) cluster of differentiation; (CAR) Coxsackievirus and Adenovirus receptor; (DAF) Decay-accelerating factor; (αvβ1,αvβ3,αvβ5) integrins; (ACE2) angiotensin-converting enzyme 2.

Briefly, the immunological cascade in myocarditis appears to initiate with the involvement of innate immune system cells. This process involves encapsulating viral compounds and deceased cells as pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), respectively. These components interact with specialized membrane receptors known as pattern recognition receptors (PRRs)[24,25]. Among these receptors, the NOD-like receptor subtype 3 (NLRP3) is particularly well-described and implicated in the inflammatory disease process[18]. Cellular injury prompts non-specific markers like reactive oxygen species to upregulate NLPR-3 and similar receptors, which identify antigens, whether exogenous or endogenous [e.g., Toll-like receptors (TLR)], ultimately functioning as inflammasomes. This multimeric protein complex assembles in the cytosol and activates the dormant pro-caspase-1, an enzyme responsible for cleaving pro-interleukin (IL)-1β and IL-18, as well as an inducer of pyroptosis[18,26,27]. In turn, IL-1β activates the innate immune system by recruiting specific cells to the site of inflammation and modulating the adaptive immune system. Furthermore, IL-18 triggers the synthesis of interferon and enhances the activation of natural killer (NK) cells, thereby augmenting major histocompatibility complex antigens on myocytes and eliminating infected cells. In the later stages of this process, T-lymphocytes are also activated by IL-18. Both CD4+ and CD8+ T cells respond to the infected cardiac cells through the complement system, antibody-dependent toxicity, and direct injury *via* T cytotoxic cells[18,27].

Human inflammatory caspase-4 and caspase-5, through a non-canonical, dependent caspase-1 activation pathway facilitated by their Caspase-Associated Recruitment Domain (CARD), are capable of autonomously activating pro-1β and IL-18[28]. Simultaneously, while inflammasomes are activated to eliminate infectious pathogens, another pathway that contributes to this process emerges pyroptosis, an inflammation-induced programmed cell death[24,27]. Conversely, caspase-1 can facilitate the pyroptosis process in a canonical manner within infected cardiac cells, engendering the creation of pores and the suppression of mitochondrial action potential *via* gasdermin D (GSDMD), a pyroptotic factor that is significantly upregulated in both epithelial and inflammatory cells[27]. These pores lead to the influx of ions and water into the cell, eventually causing a cellular rupture and releasing an abundance of cytoplasmic contents, including proinflammatory factors, alarmins, DAMPs, and nuclear and mitochondrial DNA. Additionally, GSDMD potentially plays a role in binding to cardiolipin (CL), contributing to the formation of pores on cell membranes, as well as on the inner endosome and phagosome membranes[27,28]. Finally, to facilitate inflammation resolution, certain regulatory factors are indispensable. Modulating inflammasome activation necessitates phosphoinositide-3-kinase (PI3K) to function within a negative feedback loop. Similarly, IL-1 receptor-associated kinase-M (IRAK-M) and suppressor of cytokine signaling-1 (SOCS-1) contribute by dampening Toll-like receptor (TLR) signalling[29].

Autoimmune and Auto-Inflammatory Theories

From a physiological perspective, the inflammatory pathway facilitated by profibrotic signaling culminates in the healing process through replacement fibrosis. However, in some cases, this process can evolve into a chronic autoinflammatory or autoimmune state, resulting in myocardial scarring and progressive heart failure[15,30]. While autoimmunity's involvement has been explored in murine models, research in humans remains limited[16]. Autoimmunity, understood as the outcome of self-tolerance loss, results from an imbalance between pro- and anti-inflammatory factors within both innate and adaptive immune systems. Recent studies[22,30] have unveiled interconnected genes that may partially explain myocardial autoimmunity pathways. These genes encompass TLR-signalling adaptor *Myd88*, cytokines and IL-4, chemokines, and their receptors, complement C3, and complement receptor Cr2.

Conversely, when the immune system fails to effectively clear viral material, significant viral loads persist within the myocardium, leading to a chronic, low-grade, local inflammation. In this context, T-helper (Th)1 cells play a pivotal role, acting as a primary molecular factor contributing to the chronic inflammation response[29]. The Th1 proinflammatory pathway, mediated by IL-12 and gamma interferon (IFN-γ), drives myocardial inflammatory infiltrate. This process is modulated by INF-γ synthesis, a phenomenon altered in certain murine models with defective T-Box transcription factor *TBX21* (T-bet) mice. T-bet prompts the development of Th1 lineage from naive Th1 precursor cells, both by activating Th1 and repressing opposing Th2 and Th17 genetic programs. Furthermore, the diminished response

of Th2 cells results in deficient pro-healing signals[29], a susceptibility observed in those developing autoimmune myocarditis[13,22].

Furthermore, the Th2 pathway, mediated by IL-4, is involved in specific types of autoimmune myocarditis characterized by predominant eosinophil presence. Similarly, Th17 exerts its effects through IL-17 expression, playing a crucial role in heart failure progression rather than cardiac inflammation[25]. A study[31] demonstrated that CD4+ T cells, stimulated by an initial transient IL-23 signal, are essential for establishing cardiac autoimmunity. Without sustained IL-23 action, autoreactive T cells suppressed IL-17A production.

The persistence of inflammation may continue despite effective viral clearance, as circulating antibodies could serve as inflammatory triggers, as evidenced in biopsy-proven myocarditis[32]. Moreover, B-cells are activated by dendritic cells that present viral antigens, contributing to humoral immunity by producing specific antibodies. In certain high-risk patients, B-cells may generate autoantibodies targeting shared epitopes or self-cell proteins like β1-adrenergic receptor, cardiac myosin, troponin, mitochondrial compounds, and Na/K-ATP-ase[22].

Finally, several studies[3,15,22] underscore the role of B-cells in sustaining myocardial autoimmunity, identifying immunoglobulin (Ig) G and IgG3 subclasses (potent triggers for complement cascade activation following myocyte lysis) in patients with dilated cardiomyopathy and end-stage heart failure.

Etiological Diagnosis Tools

Endomyocardial Biopsy

EMB stands as the definitive diagnostic method for myocarditis[1]. The European Society of Cardiology (ESC), guided by an expert consensus group on myocardial diseases, advocates for EMB application in all patients suspected of myocarditis. However, it is acknowledged that in the pediatric population, diagnosis remains largely clinical due to concerns regarding the potential risks of the procedure[1]. In the late 1980s, the Dallas criteria emerged as a tool to furnish a histopathological depiction of myocarditis. These criteria encompassed the presence of inflammatory infiltrates, primarily lymphocytes and macrophages, along with concurrent myocyte necrosis or damage not indicative of an ischemic event. These criteria serve as a basis for diagnostic determination[33]. Standard histological examination is a qualitative approach that introduces significant interobserver variability. Immunohistochemistry, when compared to histology alone, offers heightened sensitivity in myocarditis diagnosis[34]. It enhances diagnostic accuracy by quantifying the number of leukocytes per square millimeter and characterizing inflammatory cells, particularly those bearing CD markers. Additionally, immunohistochemistry holds prognostic value[35,36]. Moreover, the inclusion of nested real-time polymerase chain reaction (rt-PCR) to detect viral genomes in EMB samples contributes further to diagnostic capabilities[15], as evidenced in Figure 3.

EMB diagnostic biases can arise due to the uneven distribution of inflammation across different myocardial regions. Notably, the left ventricular lateral free wall is more frequently affected than the more accessible right ventricle, potentially leading to sampling bias. To address this, the utilization of low-amplitude electrocardiograms has been proposed to better guide biopsy toward affected myocardial areas[37]. Despite the perceived risks associated with EMB, a considerable cohort of German pediatric patients has shown a different perspective. The ESC Working Group for Myocardial and Pericardial Disease advocates for an increased application of EMB in the pediatric population[38]. In such cases, the utilization of immunochemistry on EMB specimens and the accuracy of PCR in detecting viral genomes offer valuable insights crucial for accurate diagnosis and early therapeutic management[38]. Furthermore, three cohorts studies[39-41] have reported that major adverse events stemming from the procedure occurred in 2.6%[39], 5%[40], and 13.2%[41] of biopsied infants with suspected cardiomyopathy. A less invasive alternative to EMB entails conducting PCR analysis on tracheal aspirate samples in young patients with myocarditis. Interestingly, the EMB specimens showed similarities with those collected from the respiratory tract[42]. Nonetheless, the utilization of biopsy remains confined to carefully selected cases within the pediatric population[1].

Immunology Viral Straining

Viral genomes play a prominent role in the etiology of myocarditis[17,36]. Among these, notable culprits include enteroviruses, particularly coxsackievirus, adenovirus, parvovirus B19, human herpesvirus 6, Epstein Barr virus, and the more recent addition of SARS-CoV-2[19,21,22].

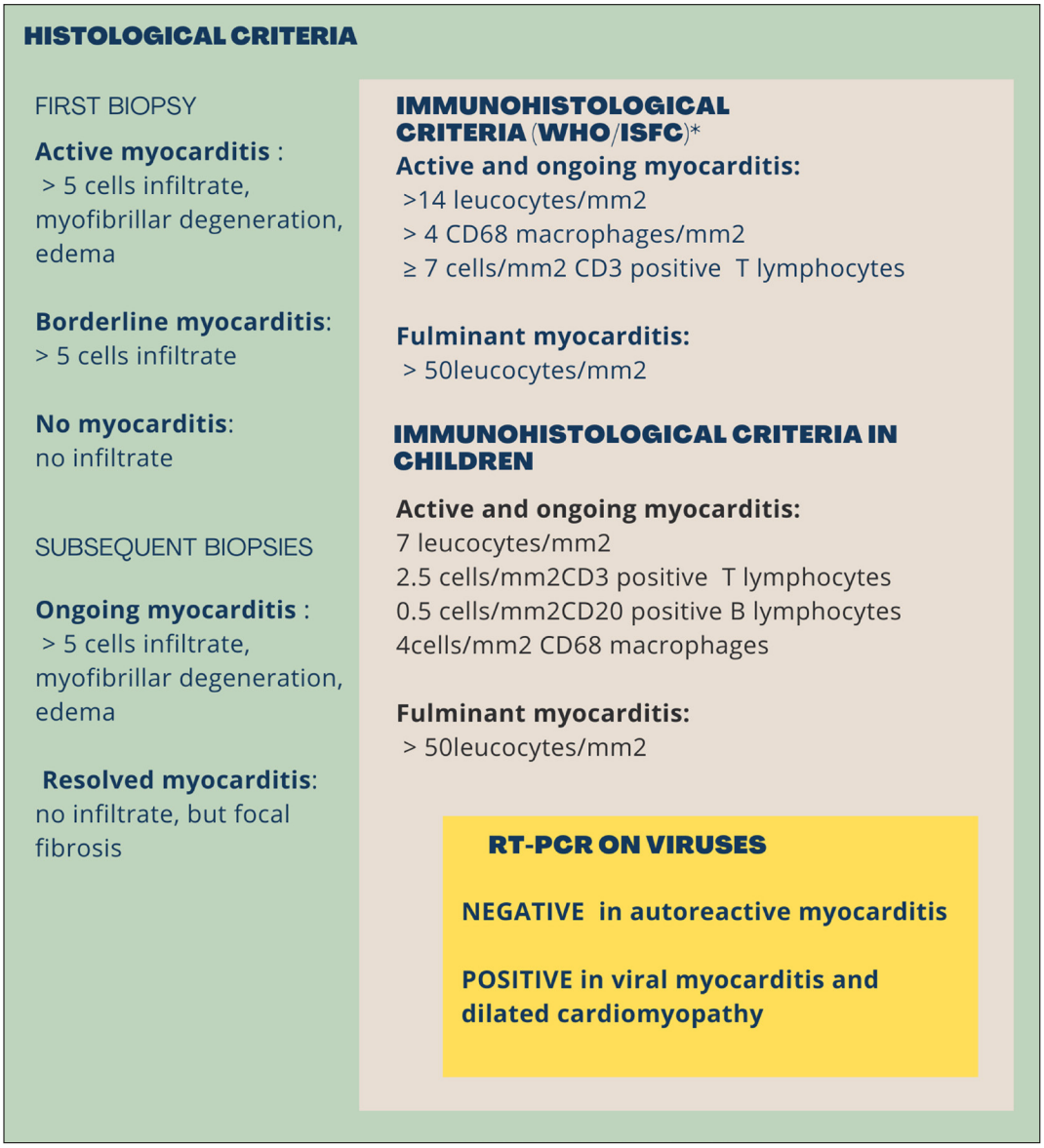

Figure 3. Comparison of histological, immunohistochemical criteria and the predictability of rt-PCR on viral genome in EMB- patients[11,12,25,36]. (WHO) World Health Organization, (ISFC) International Society and Federation of Cardiology.

Despite the diversity of these viruses, they converge on a common pathogenic autoimmune process, resulting in chronic inflammation and tissue remodeling that detrimentally impacts left ventricular systolic function[21]. Establishing effective treatment strategies and refining diagnostic tools are critical endeavors. Former techniques like *in situ* and slot blot hybridization have fallen out of favor due to their tendency to yield false positive results. Instead, emphasis has shifted towards enhancing the value of EMB through amplification methods[43]. Presently, viral etiologies of acute or chronic cardiomyopathies are substantiated through viral genome analysis using quantitative PCR methods, including real-time PCR and nested PCR with reverse transcription. These techniques facilitate the detection of low copies of viral RNA or DNA sequences from endomyocardial samples[22,44], although it is important to note that conventional PCR might not detect mutant species[25]. While a positive PCR outcome is diagnostic, corroborating viral serology from peripheral blood obtained at the time of EMB is crucial to exclude the possibility of passive blood contamination. However, a negative PCR result

does not definitively rule out viral disease, as it may be influenced by sample size bias[43]. The presence of cardiac autoantibodies indicates an immune-mediated process and highlights the suitability of immunosuppressive therapy[36]. Utilizing PCR analysis, including assessing viral replication status and viral load, is advised for follow-up biopsies in patients with viral myocarditis to evaluate the efficacy of specific antiviral therapy[36,43]. A key advantage of PCR analysis lies in its capability to detect both latent and active infections, addressing etiologies that may be more elusive using conventional methods. Furthermore, it enables strain typing, detection of virulence factors, and determination of antimicrobial resistance[45].

Biomarkers

Currently, there exists a consensus on the utility of nonspecific circulating biomarkers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) to indicate the systemic inflammatory status during the acute phase of myocarditis. However, these biomarkers lack specificity in terms of diagnosis, prognosis, or risk stratification[1]. In contrast, troponins are more specific indicators of myocardial damage, particularly in cases of acute coronary syndrome, and hold significant prognostic value[32]. B-type natriuretic peptide (BNP), synthesized from its precursor N-terminal pro b-type natriuretic peptide (NT-proBNP) under physiological conditions, is released into the bloodstream when systolic or diastolic dysfunction occurs. Nonetheless, these markers exhibit low negative predictive values[5,32]. Throughout the three-phase progression of myocarditis, following the acute phase, a disrupted healing process leads to necrosis evolving into extensive fibrosis, clinically manifested as heart failure or sudden cardiac death[1,22,25]. Myocardial fibrosis plays a pivotal role in the cardiac remodeling process, characterized by excessive stimulation of fibroblasts and augmented production of the extracellular matrix (ECM), coupled with cardiomyocyte apoptosis[29]. At the cellular level, persistent cardiac injury, regardless of its origin, triggers the release of various substances that propel the progression toward fibrosis[29,46,47]. Among the various cell types constituting the myocardium, cardio fibroblasts play a critical role in maintaining cardiac ECM homeostasis and remodeling. They contribute to processes like angiogenesis, cell proliferation, cardiomyocyte hypertrophy, and apoptosis[47,48]. Importantly, these cells can differentiate into myofibroblasts (myoFBs), which perpetuate the inflammatory response to injury by producing cytokines such as IL-1α, IL-1β, IL-6, IL-10, and tumor necrosis factor-alpha (TNF-α). Additionally, myoFBs exhibit an enhanced synthetic capacity for ECM proteins[49].

During the pathological process of ECM remodeling, the degradation of collagen fibers results in the cleavage of telopeptides located at the amino-terminal or carboxy-terminal ends of collagen molecules. This cleavage leads to the release of pro-peptides, specifically the amino-terminal propeptide of type I procollagen (PINP) and carboxy-terminal propeptide of type I procollagen (PICP) for collagen type I, and the amino-terminal propeptide of type III procollagen (PIIINP) and carboxy-terminal propeptide of type III procollagen (PIIICP) for collagen type III. These pro-peptides are liberated during the biosynthesis of these collagen types in a balanced manner and are thus regarded as biomarkers of collagen synthesis. Conversely, biomarkers of collagen degradation are generated when collagen type I (CITP, NITP) and type III (CIIITP, NIIITP) are broken down, a process that occurs during ECM degradation[48]. In a physiological context, collagen is stable and resistant to most proteinase activity, apart from matrix metalloproteinases (MMPs), which are implicated in cardiac remodeling. The resistance or cross-linking of collagen type I fibers is indicative of the slower cleavage of the carboxy-terminal telopeptide of collagen type I (CITP) by the enzyme MMP-1. This interplay results in the serum CITP: MMP-1 ratio displaying an inverse correlation with myocardial collagen cross-linking. Therefore, maintaining a balance between the action of MMPs and the activity of their inhibitors (TIMPs) is crucial in modulating myocardial fibrosis[46,47,50].

Research efforts have concentrated on assessing the predictive significance of the three established biomarkers - PINP, PIIINP, and CITP: MMP-1 ratio - and their correlation with myocardial collagen fraction in patients afflicted with advanced-stage heart failure and dilated cardiomyopathy, which often arise as late complications of myocarditis[47,50]. Transforming growth factor β (TGF-β), a signaling cytokine secreted by diverse cell types, including macrophages, assumes a pivotal role in various disease contexts[51,52]. In the realm of cardiac pathology, it functions as a mediator of remodeling, prompting fibroblast differentiation and driving myocyte apoptosis.

TGF-β engages the TGF-β type II receptor to initiate the *Drosophila* gene "*mothers against decapentaplegic*" *Caenorhabditis elegans* small protein (SMAD)-signaling pathway. Subsequently, the formed protein complex traverses into the cell nucleus, functioning as a transcription factor that orchestrates genes implicated in apoptosis *via* the mitogen-activated protein kinase 8 pathway. The SMAD pathway is governed by feedback regulation. Markedly elevated circulating levels of TGF-β have been detected in patients with dilated cardiomyopathy[46,51,52]. Furthermore, emerging evidence indicates that endoglin and fibulin 2, ECM proteins, function as co-receptors for TGF-β, exerting a profibrotic influence on cardiac fibroblasts and modulating TGF-β1's impact on extracellular matrix synthesis. Caveolins, a group of membrane proteins, are involved in receptor-independent endocytosis. In myocardial cells, caveolae-3 (Cav-3) serves as a scaffolding protein. Studies[53] in murine models have shown that the expression of these proteins is linked to the prognosis and survival rates. These markers have garnered attention due to their potential as therapeutic targets, not solely for diagnosing cardiac fibrosis[46,54,55]. Galectin-3 (Gal-3), a soluble beta-galactosidase-binding lectin belonging to the galectin family, plays a pivotal role in host defense against injury and in the modulation of the transition towards chronic inflammation, ultimately favoring fibrosis. Extensive animal studies[56-58] have illuminated Gal-3's involvement in myocardial fibrosis, triggering the activation of cardiac fibroblasts by modulating TGF-β's function. Gal-3's overexpression is mediated by the profibrotic influences of aldosterone and angiotensin II, mechanisms contributing to the progression of heart failure. While Gal-3 carries informative value, it is important to note that it may serve as a marker for confirming the cardiac inflammatory process and fibrosis, dependent on the etiology of heart failure. However, circulating Gal-3 concentrations do not appear to accurately reflect endomyocardial Gal-3 levels or cardiac fibrosis[45,56]. Recent studies[59-61] have proposed several potential diagnostic or prognostic indicators in cardiovascular disease, although they do not specifically target the diagnosis of myocarditis. Among these, notable mentions include growth differentiation factor-15 (GDF-15) and pentraxin 3. GDF-15, also known as macrophage inhibitory cytokine-1, belongs to the TGF-β cytokine superfamily. In physiological contexts, it exhibits high expression in placental cells, and a more subdued presence in cardiomyocytes and other cell types. Elevated GDF-15 levels have been associated with inflammation and have been noted to be upregulated by factors like IL-1β, TNF-α, and IL-2, particularly in peripheral tissues of patients with heart failure and dilated cardiomyopathy. Pentraxin 3 (PTX3), a soluble pattern recognition molecule, falls within the pentraxin superfamily alongside C-reactive protein. Its expression is induced by IL-1β, TNFα, and pathogenic compounds originating from cells such as macrophages, neutrophils, and fibroblasts. Conversely, high-density lipoproteins (HDL) and IL-10 act as down-regulators for PTX3. Endothelial cells contribute to PTX3 release, affording it a protective role. Operating through the regulation of complement activation, pathogen opsonization, innate immunity, and inflammation, PTX3 plays a crucial part in an extracellular matrix organization and cardiac remodeling facilitated by MMPs. Elevated circulating levels of PTX3 appear to correlate with severe heart failure and unfavorable outcomes, thereby positioning this molecule as a potential prognostic factor[62-64]. Suppression of tumorigenicity 2 protein (ST2), a member of the IL-1 receptors family, has emerged as a key player in inflammation and tissue fibrosis[65]. ST2 acts through its ligand, IL-33, which activates a nuclear signal pathway, triggering an immunomodulatory response in tumor cells, as well as in heart and kidney cells. This interplay carries significant implications in these tissues, with potential consequences for inflammation and fibrosis processes[66].

MicroRNAs

The pursuit of identifying informative biomarkers for the diagnosis and prognosis of various diseases, including the contentious area of acute myocarditis, has led to the discovery of novel RNA fractions associated with genetic, molecular processes. This journey was catalyzed by a seminal scientific breakthrough in the late 1980s involving the study of gene expressions of lin-4 and lin-14 in the temporal development of *Caenorhabditis elegans* larvae. This endeavor illuminated the existence of micro-RNAs (miRs), distinct genetic elements that do not encode proteins but instead interact with untranslated regions (5'UTR or 3'UTR) of messenger RNAs (mRNAs), thereby modulating the expression of specific genes in both physiological and pathological contexts[67-72]. The prominence of miRs in the intracellular and extracellular milieu, cou-

pled with their remarkable stability even under extreme conditions such as temperature or pH variations, has positioned them as desirable biomarkers for the diagnosis, progression, and prognosis of diverse diseases[71,73]. Among cardiac tissue-specific miRs, certain molecules act as up-regulators (e.g., *miR208*) or down-regulators (e.g., *miR126*), and their roles have been more comprehensively elucidated in patients with coronary artery disease[73]. In the acute phase of myocarditis, as previously mentioned, Th17 cells contribute to the humoral immune response, and elevated levels of Th17-derived miRs have been observed, such as *Mmu-miR-721* in murine models and *has-miR-Chr8:96* in human myocarditis patients[74,75]. Several cardio-miRs are implicated in shaping the acute myocarditis process. *MiR-21-5p* and *miR-208a*, both connected with left ventricular systolic dysfunction, serve as prognostic indicators for recovery during the late stages of acute myocarditis[76,77]. *MiR-1-3p* is associated with the extent of myocardial damage, mirroring troponin I levels[77]. Further, the over-expression of *miR-30a* and *miR-181d* can alter the immune response to Coxsackievirus B3 by inhibiting SOCS-3[78]. Conversely, a beneficial outcome was observed with the upregulation of both *miR-155* and *miR-148a*. Increased *miR-155* levels can effectively modulate the immune response to Coxsackievirus B3 by suppressing the nuclear signaling pathway, ultimately leading to improved survival rates among mice infected with the virus[79]. Moreover, the diagnostic accuracy of acute myocarditis has been enhanced by the elevated expression of *miR-146b*, in correlation with cardiac troponin I, serving as an antigen (anti-cTNI)[80]. Recent findings[81] introduce a novel approach, suggesting a serum exosome miR panel for molecular diagnosis of myocarditis. Evaluating the circulating plasma levels of specific miRs, such as *has-miR-30a*, *has-miR-192*, *has-miR-146a*, *has-miR-155*, and *has-miR-320a*, collectively demonstrates higher diagnostic significance than individual miR assessment. This panel, especially characterized by *has-miR-155* and *has-miR-320*a, offers superior differentiation between fulminant and non-fulminant myocarditis forms[81]. Beyond their diagnostic utility, cardiac miRs in acute myocarditis also hold prognostic significance and offer potential avenues for targeted treatment strategies. In summary, Table II provides an overview of the specificity and predictive value of biomarkers in the diagnosis of myocarditis.

Therapy

The diagnosis of myocarditis remains a complex challenge, and the treatment options are subject to ongoing debate. The diagnostic landscape continues to evolve as researchers explore various methods. Despite the gold standard status of EMB and PCR-related tests for diagnosing myocarditis, a significant number of cases within the pediatric population lack a clearly identified etiology. This underscores the need for improved diagnostic strategies that can provide more definitive answers. Moreover, the course of myocarditis can vary widely, with outcomes ranging from remission to unpredictable or unfavorable trajectories. This uncertainty underscores the importance of adopting a broad and supportive approach to management, especially given the lack of specific treatment options. As research advances and our understanding of myocarditis deepens, the hope is that more effective diagnostic methods and targeted therapeutic interventions will emerge, enhancing the care and outcomes of patients affected by this complex condition.

Symptoms-Based Therapy

In the absence of well-established treatment guidelines specifically tailored for pediatric patients with myocarditis, current strategies largely revolve around managing heart failure and arrhythmias, which are common manifestations of the condition. Beta-blockers and diuretics have shown promise in animal studies as beneficial interventions for managing heart failure in acute myocarditis patients[82-84]. Carvedilol, for instance, has demonstrated cardioprotective effects in autoimmune myocarditis by acting as an antioxidant and downregulating the synthesis of inflammatory cytokines[84]. In terms of diuretics, torsemide has shown superiority over furosemide, primarily by improving left ventricular function and delaying the process of cardiac remodeling, which often leads to dilated cardiomyopathy[83]. Arrhythmias are another significant concern in patients with suspected myocarditis, and they may manifest as sinus bradycardia, QRS complex prolongation, atrioventricular blocks, or tachyarrhythmias. Subacute phases of myocarditis can even present with ST-wave changes, further highlighting the electrical instability associated with the condition[1,38]. The underlying mechanisms of arrhythmias can be multifactorial, encompassing structural changes due to viral invasion, ischemia secondary to endothelial tropism of viruses like

Table II. Specificity and predictive value of biomarkers in risk stratification of myocarditis.

Pathway	Biomarkers	Key Role	Specificity	Predictive value	References
Nonspecific	ESR CRP	Systemic inflammation	- -	- -	[1]
Specific	Troponin BNP, proBNP	Acute stress myocardial destruction Myocardial dysfunction	+ +	+ -	[5,32]
Collagen	PINP, PIIICP CITP, NIIITP CITP: MMPs	Collagen synthesis Collagen degradation Collagen degradation	+ + +	+ + +	[47,82]
Fibrosis	TGF- β Fibulin -2	Fibroblastic differentiation TGF β- co-receptor	- -	+ +	[46,51,52]
Inflammation and fibrosis	Galectin-3 Growth differentiation factor -15 PTX3	 IL-1β, TNF-α, IL-2- stimulation Complement opsonization	- - -	+ + +	[45,56] [59-61] [62-64]
Gene expression	*has-miR-Chr8:96* *miR-21-5p, miR-208* a Panel *has-miR-30a-has-miR-192-has-miR-146a-has-miR-155-has-miR-320a*	Acute process Left ventricle dysfunction Fulminant *vs.* non-fulminant myocarditis	+ + +	+ + +	[75,77] [76,80] [81]

(ESR) erythrocyte sedimentation rate; (CRP) C-reactive protein; (BNP) brain natriuretic peptide; (PINP) P-terminal propeptide of procollagen type I; (PIIICP) C-terminal propeptide of procollagen type III; (CITP) C-terminal propeptide of procollagen type I; (NIIITP) N-terminal propeptide of procollagen type III; (MMPs) Matrix metalloproteinases; (TGF- β) transforming growth factor beta; (TNF) tumor necrosis factor; (PTX3) Pentraxin 3.

Parvo B19, disruption of gap junctions and connexins, direct effects on calcium ion channels, and fibrotic scar formation[85]. Given the persistent risk of arrhythmia development, even beyond the acute phase of the disease, antiarrhythmic medications are generally considered for these patients. Rigorous follow-up is crucial, particularly in the first year post-diagnosis. In cases where there is an increased risk of sudden death, consideration may be given to implanting a cardiac device or using temporary interventions like wearing a Life Vest to bridge transient cardiac rhythm disturbances[1,22]. While there is a lack of standardized therapeutic protocols for pediatric myocarditis, these approaches aim to mitigate heart failure symptoms and manage arrhythmias, thereby improving patient outcomes.

Etiology Proven-Based Therapy

In pediatric populations, determining the precise etiology of myocarditis can indeed be challenging, and reliance on peripheral blood serology is common due to the invasive nature of endomyocardial biopsy. However, when a viral cause is confirmed, targeted therapies can offer effective treatment options. For viral-induced myocarditis, interferon (IFN)β therapy has shown promising results. In cases of adenovirus or enterovirus-induced myocarditis, IFNβ therapy has been associated with enhanced viral clearance and improved left ventricular ejection fraction[86,87]. It is important to note that the efficacy of IFNβ therapy can vary depending on the specific viral agent causing the myocarditis. For instance, while high doses of IFNβ have been found to improve endothelial function in Parvo B19-positive myocarditis cases, they may not lead to efficient viral clearance[88]. In cases where myocarditis is caused by viruses like EBV, CMV, and HHV-6, antiviral drugs like acyclovir, ganciclovir, and valacyclovir can be considered as treatment options[20,22,36,89].

These medications target the specific viruses causing myocarditis and aim to reduce viral replication and associated inflammation. In addition to the mentioned therapies, other antiviral drugs like pleconaril and pocapavir have shown efficacy in decreasing cellular alterations in cases of enteroviral-induced myocarditis[38,90-92]. These treatments are designed to directly impact the viral replication and associated effects on the heart tissue. It is important to emphasize that treatment decisions should be based on a comprehensive evaluation of the patient's clinical condition, the specific viral etiology, and potential benefits and risks associated with each therapy. As research continues to advance, more targeted and effective therapies may become available for the management of myocarditis in pediatric patients.

Pathogenesis-Based Therapy

Immunosuppression therapy, often involving prednisone or prednisolone, either alone or in combination with medications like azathioprine or cyclosporine, has shown promise in improving left ventricular ejection fraction in cases of virus-negative myocarditis[1,25,93,94]. These therapies work by suppressing the immune response that contributes to the inflammatory process and cardiac damage. Intravenous immunoglobulin (IVIG) is indeed an important immunomodulatory treatment option. It contains a collection of antibodies obtained from healthy donors, which exert various effects on the immune system. The mechanisms through which IVIG exerts its therapeutic effects are complex and involve both fragment antigen-binding (Fab)-dependent and fragment crystallizable (Fc)-dependent pathways. Fab-dependent mechanisms involve interactions with B-cells and complement factors, leading to the suppression of autoreactive clones and inflammation[3,95]. Fc-dependent mechanisms involve interactions with macrophages, regulatory T-cells, and other immune cells, resulting in the modulation of immune responses and suppression of inflammation[3,96]. The multifactorial immunomodulatory effects of IVIG extend beyond its antiviral properties. It has been shown to neutralize pathogens and inhibit inflammatory cytokines, contributing to its anti-inflammatory effects. Recent data[97] from a Cochrane metanalysis further support the use of IVIG in patients with acute myocarditis, both in children and adults. This analysis highlighted the beneficial effects of IVIG treatment, showing improved left ventricular ejection fraction in patients who received IVIG therapy compared to those who did not. For cases of fulminant myocarditis with a rapidly deteriorating clinical course, non-therapeutic options such as circulatory mechanical support and heart transplantation become crucial to providing life-saving interventions and addressing severe cardiac impairment. Overall, the approach of immunomodulation, including IVIG, represents an important therapeutic avenue in cases of non-viral proven or auto-reactive myocarditis, aiming to mitigate the immune response and its damaging effects on the heart tissue.

Futures Perspectives

These potential therapies offer innovative approaches to targeting specific pathways and mechanisms involved in the inflammatory and fibrotic processes associated with myocarditis. Immunoadsorption followed by intravenous immunoglobulin administration is an interesting approach to address autoantibodies, such as beta (1)-adrenergic receptor (β(1)AR) autoantibodies, that contribute to cardiac inflammation. By removing these autoantibodies and modulating the immune response with IVIG, this treatment strategy aims to reduce inflammation and improve left ventricular function[36,98,99]. Aldosterone antagonists like eplerenone, which have been used in other cardiac conditions, are being explored for their potential benefits in cardiac remodeling by targeting mast cell gene expression and reducing inflammation[100]. Targeting specific cytokines has also gained attention. Inhibiting IL-1β with anti-mouse IL-1β antibodies has shown promise in reducing inflammation and fibrotic scar formation, potentially preventing aberrant cardiac remodelling[101]. Similarly, Secukinumab, an anti-IL-17 monoclonal antibody, is being considered to interrupt the profibrotic pathways induced by IL-17 in autoimmune cardiac impairment[102]. The use of antisense miRNA complements (antagomirs) as targeted therapy is a novel approach in autoimmune myocarditis. By administering *antagomiR-21a-5p*, researchers were able to attenuate myocardial inflammation and fibrosis in experimental models of induced myocarditis[103]. This highlights the potential of miRNA-based therapies in modulating gene expression associated with pathological pro-

Table III. Proposed therapy in AM, including accepted and perspective therapies, aims and accomplishments.

Therapy	Target	Drug class/medication	Role	References
Symptoms-based	Heart failure arrhythmias sudden death risk	Beta-blokers (Carvedilol) Diuretics (Torsemide) Anti-arrhythmics	Antioxidant cardiac remodeling	84 83 1,85
Aetiology proven-based	Parvo B-19, EBV, CMV, HHV-6 Enteroviruses SARS-CoV 2	IFNβ Antivirals (Acyclovir, Ganciclovir,Valacyclovir) Pleconaril, Pocapavir Remdesivir	Improve viral clearance Improve left ventricular function	20-22,36,86,89 38,90-92 23
Pathogenesis-based	Immuno-modulation	Prednisone, Prednisolone IVIG	Improve left ventricular function	1,25,93,94 97
Perspectives	Inflammatory/ Fibrotic process	Immunoadsorbtion + IVIG fibrotic process Aldosterone antagonist (Eplerenone) Anti-mouse IL-1β antibodies Anti-IL-17 monoclonal antibodies (Secukinumab) Ulinastatin + creatine phosphate sodium antisense miRNA complements (*antagomir-21a-5p*)	Cardiac remodelling IVIG	36,98,99 100 101 102 104 103

cesses. Ulinastatin, an acid-resistant protease inhibitor naturally present in human urine, has gained clinical recognition across various diseases due to its robust anti-inflammatory properties, making it a viable alternative to steroids. Recent research[104] has revealed its remarkable protective impact on cardiomyocytes, significantly improving cardiac function and reducing myocardial injury, especially when combined with creatine phosphate sodium. These findings hold promising implications for the treatment of viral myocarditis in pediatric patients. Ongoing human trials are crucial to validate the efficacy and safety of these emerging treatments and to further refine their therapeutic potential. These perspectives hold promise for providing more targeted and effective therapeutic options for patients with myocarditis, addressing the underlying immune and inflammatory mechanisms while aiming to improve cardiac function and patient outcomes. Table III summarizes the accepted treatment and prospects for future therapies in acute myocarditis.

Conclusions

The diagnosis of myocarditis involves various methods, with endomyocardial biopsy serving as the gold standard for confirmation. However, due to the risks involved, especially in pediatric patients, other diagnostic tools like immunohistochemistry and rt-PCR play a significant role in investigating viral etiology in myocardial samples. Biomarkers have emerged as promising candidates for both diagnostic and prognostic purposes, helping clinicians assess the inflammatory and cardiac remodeling processes. These markers can offer insights into disease severity, progression, and treatment response. Understanding the underlying pathogenesis of myocarditis is crucial for tailoring effective treatments. Different etiologies, such as viral infections or autoimmune reactions, drive distinct inflammatory and immune responses. Targeted therapies that focus on specific pathways or immune components associated with these etiologies have shown potential for better outcomes. Research efforts are ongoing to uncover more precise diagnostic methods, ef-

fective therapeutic interventions, and a deeper understanding of myocarditis complexities. This comprehensive approach will ultimately contribute to improved patient care, better treatment outcomes, and a more informed clinical management of this challenging cardiac condition.

Conflict of Interest
The authors declare that they have no conflict of interests.

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Authors' Contribution
L. Agoston-Coldea conceptualized the manuscript's design. A. Popa and C. Lazea significantly contributed to the manuscript's conception; A. Popa performed the literature search and drafted the manuscript, L. Agoston-Coldea and C. Lazea supervised, verified the contents, and revised the manuscript. The authors reviewed and approved the final version of the article to be published.

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Data Availability
The authors confirm that the data supporting the findings of this study are available within the article.

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